ERNST (H.C.)

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THE BACILLUS OF TUBERCULOSIS.

BY

HAROLD C. ERNST, A. M., M. D. (Harv.),

OF JAMAICA PLAIN, MASSACHUSETTS.

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THE BACILLUS OF TUBERCULOSIS.

By HAROLD C. ERNST, A.M., M.D. (Harv.),
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So many methods of observation and of staining the bacillus of tuberculosis have been suggested and recommended as possessing various advantages, that a systematic investigation of their comparative merits seemed to be necessary in order to the determination of the diagnostic value of each. By diagnostic value is meant, of course, the power of separating under all circumstances the special bacillus under consideration from other forms of bacteria. This investigation has been very thoroughly carried out, and the results are given below. These results have been obtained after the expenditure of much time in the preparation of the slides, much care in the selection and compounding of the staining reagents, and by the most careful comparison of the slides one with another. The staining materials employed have been the purest that could be obtained, having been procured in all cases direct from the packages of the importer. The microscope used has been the number 1 Zeiss stand, a number 3 ocular, a $\frac{1}{12}$th homogeneous immersion lens, and an Abbé's illuminator. Thus absolute uniformity as regards the apparatus for observation has been maintained.

Staining Methods and Materials.—Of the methods that now claim attention from the prominence of the gentlemen proposing them, or by reason of their own peculiarities, there are four—those of Gibbes, Fränkel, Ehrlich, and Koch (the latter is really that of Weigert, but, as it has been adopted by Koch, it is called by his name in this paper). These methods will be considered in detail.

I. Gibbes's methods. This proposes two methods of staining, one requiring a longer time for its completion than the other. In the first method he uses magenta for the first stain, decolorizes in nitric acid, and uses methylene blue, methyl green, or iodine green, as a contrast stain. This only differs from that of Ehrlich or Koch in the length of time

necessary for immersion in the first stain, which its author claims to be but from twenty to thirty minutes. It need only be said that in numerous cases this time has been found to be too short for the staining of the bacilli, and in not a few cases failure to detect them at all would have occurred unless one of the more reliable methods had been employed as control.

The second of this author's methods—the so-called "rapid method"—"does away with the use of nitric acid, and is a very rapid one for diagnostic purposes, as it can be accomplished in five minutes after the sputum is dried on the cover-glass." The staining can be completed as rapidly as is here stated, but exception must be taken to the statement that the method is of value for diagnostic purposes—it has no such value whatever. In this statement I am supported by Fränkel (*Berl. Klin. Woch.*, April 7 and 14, 1884).

The method has been used so often, and recommended so highly that a complete statement of it will be of value. The stain is a combination of rosanilin-hydrochloride, and methyl blue, and is prepared as follows:—

1. Take of rosanilin hydrochloride, gm. ij;
   and of methyl blue, gm. j.
   Rub well together in a mortar.

2. Of anilin oil, c.c. 3;
   Spts. rectificat. c.c. 15.
   Solve.

Add 2 to 1 slowly till all the stain is dissolved, then add slowly aq. dest. c.c. 15.

To use the stain, heat a few drops in a test-tube till steam arises, then pour into a watch-glass, and allow the cover-glass to stay in it for from four to five minutes. Wash in methylated spirit (alcohol) until no more colour comes away, dry and mount in Canada balsam. The bacilli of tuberculosis are stained red, and all others blue.

The rosanilin hydrochloride I have used was sent to me from London by Dr. Gibbes's order, as it was found to be impossible to obtain a supply in this country. There can be no doubt, therefore, that the salt used was the right one.

The two colours, the rosanilin-hydrochloride and the methylene blue, do not combine, but form a simple mixture, as may be proven by pouring a thin layer upon a watch-glass, allowing it to dry, and viewing it by transmitted light; the red and the blue colours may be seen lying side by side.

The results of a large number of experiments with this method of staining, using preparations of sputum, cultures of the bacillus of tuberculosis, and cultures of other micro-organisms, are these: 1st. The staining is likely to be very unsatisfactory and indistinct. 2d. The same organisms

may be stained red or blue indifferently upon the same field of the microscope. 3d. There are organisms, other than the bacillus of tuberculosis—as proven by their behaviour to Koch's or Ehrlich's staining method and the conditions under which they were cultivated—which stain red by this method. Still more, the fluid is so very concentrated that a drop of it in the wrong place is quite as likely to do harm as the nitric acid it is intended to dispense with. This "rapid method," therefore, seems to be absolutely worthless from a diagnostic point of view, for staining the organism for which it is employed. Its only recommendations—rapidity and the doing away with the use of nitric acid—are nothing in comparison with the objections that may be brought against it.

Fränkel (*loc. cit.*) has attempted to shorten the time necessary for obtaining a contrast stain to the bacillus of tuberculosis in sputum, and publishes a "ready method" also, which he claims to be as valuable from a diagnostic point of view as that of Ehrlich or Koch. This, I think, may fairly be disputed. His staining fluids are, in brief, as follows:—

1. ℞.—Ol. anilin, c.c. 3;
   Spts. rectificat. c.c. 7;
   Aq. dest. c.c. 90.
   M. et filtra.

This he calls his "solution of anilin oil."

2. ℞.—Fuchsin (or methyl violet), gm. 1;
   Spts. rectificat. q. s. ad sol. sat.—M.

At the time of examination add, drop by drop to the solution of anilin oil, as much of the staining fluid as is necessary to produce a strong opalescence, first warming the anilin oil solution; three minutes is sufficient for staining.

For control staining and decolorization *at the same time*, he uses one of the following solutions—the first or third, if the first stain be fuchsin, the second, if it be methyl violet:—

1. ℞.—Spts. rectificat. P. 50;
   Aq. dest. 30;
   Acid. nitric. 20;
   Methyl blue, q. s. ad sol. sat.
   M. et filtra.

2. ℞.—Spts. rectificat. P. 70;
   Acid. nitric. 30;
   Vesuvin. q. s. ad sol. concent.
   M. et filtra.

3. ℞.—Spts. rectificat. P. 50;
   Aq. dest. 20;
   Acid. acet. 30.
   Malachrite (or ethyl) green, q. s. ad sol. concent.
   M. et filtra.

After the first staining the preparations are washed in water and then placed in one of these solutions for from one to two minutes; they are then washed in water, or fifty per cent. alcohol with one per cent. of acetic acid, dried with blotting paper and then with flame, and finally mounted in Canada balsam.

I have tried this method with sputum preparations, and with cultivations of various micro-organisms, including the bacillus of tuberculosis, using Ehrlich's or Koch's methods for control.

In preparations from cultures, the results were poor, for it was found that not all the organisms were completely decolorized, and there were found occasional masses of bacteria, which could be seen to be of a distinctly red colour, and which were as distinctly different from the bacillus of tuberculosis.

In sputum preparations this quick method is exceedingly unsatisfactory, the whole field being very faintly coloured, and what bacilli were stained being so indistinct as to require the most favourable conditions of observation for their detection. In one case, this method showed very few bacilli on but one slide out of four, and these very indistinct. The second cover glass from each of these, stained after Ehrlich's method, showed enormous numbers of the red rods scattered everywhere over the field.

The objections to this method seem to be, that there is not sufficient time allowed for the first staining of the specimen, nor for its decolorization; and that the decolorizing agent is not sufficiently strong, nor is the contrast stain allowed to act for a sufficient length of time.

The third method of staining, that of Ehrlich's, in which the colours employed are fuchsin and methylene-blue, and the decolorizing agent is nitric acid, and in which twenty-four hours is needed for its completion, is so well known that extended comment upon it is unnecessary. It will not be out of place, however, to add the writer's testimony that, after an experience extending over hundreds of examinations, and including many forms of bacteria, he has never found any micro-organism but the bacillus of tuberculosis that will resist the decolorization with nitric acid as employed in this method.

The last method to be mentioned in detail is that now employed by Koch, and given in full in the last volume of the German health reports. (*Mitt. a. d. Kais. Gesundheitsamte*, Bd. II. S. 10, 1884.) It is as follows:—

Sections should be made from preparations well-hardened in alcohol. Stain with a solution thus prepared:—

℞. Anilin water (sat. sol.), c.c. 100;
Alcoholic solution, methyl violet (or fuchsin), c.c. 11;
Absolute alcohol, c.c. 10.—M.

(The anilin water is made by thoroughly shaking a few drops of anilin oil with distilled water, until no more is dissolved, and filtering.)

The preparations should be left at least twelve hours in this solution (cover-glass preparations may be stained quicker by warming the fluid).

At the end of twelve hours put the preparations in dilute nitric acid (one part to three of distilled water) for one second.

Wash in sixty per cent. alcohol for one minute (cover-glass preparations are sufficiently washed by merely passing to and fro).

Stain again with a solution of vesuvin (of such strength that a layer 2 (two) c.c. thick is barely transparent), or of mythelene-blue (for contrast to fuchsine) for one minute.

Wash first in sixty per cent. alcohol, and lastly in absolute alcohol.

Mount in oil of cloves or Canada balsam for those which are to be preserved.

As will be seen, the main difference between this and Ehrlich's method is the employment of alcohol for washing the preparations, and the shorter time in which they are left in nitric acid. Koch thinks that the acid has done its work in a moment, and that the superfluous colour then remaining is to be best removed by alcohol rather than by a longer immersion in the nitric acid. Adopting the methyl-violet and vesuvin colours, the bacilli will be found blue and the ground substance brown, and it is this contrast which Koch seems to prefer.

In my experiments with this method, which have extended over preparations of tissue, sputum, pure and impure cultures of the bacillus of tuberculosis, and of other organisms, I have obtained decidedly better results than with any other. The advantages over Ehrlich's method are the shorter time of immersion in nitric acid, thus producing less distortion of the sections, and the shorter time in which the contrast staining and mounting can be completed. This latter is a very great recommendation when a large number of specimens are being prepared at the same time. The advantages of the use of the violet stain with vesuvin as a contrast, as far as it has been manifest to the author, is that the bacilli of tuberculosis are brought out more clearly, and the details of their structure made very much more apparent—the spore formation being as distinctly visible as in much larger organisms stained by other methods. Indeed, the writer must confess to never having really seen the shape of spores in these minute rods until these colours were employed. The same high praise cannot be given to these colours when used in the examination of material from tissue for purposes of diagnosis. The contrast given by the vesuvin is not sufficiently sharp, and the discovery of single bacilli scattered very sparsely through a preparation is much more difficult than when fuchsin and methylene-blue are used.

All of these methods have been judged more especially with regard to their value from a diagnostic point of view, and the endeavour has been conscientiously made to discover if any other organism than the bacillus of tuberculosis is affected in the same way by them. Of course, the value

of any method of staining depends in the first place upon its power of isolating the special bacillus under consideration, and, in the second place, upon its power of doing this clearly, certainly, and quickly. No shortening of the time necessary, or quickness and ease of manipulation is of any value, if by employing them we lose what seems to be the especial strength of Koch's or Ehrlich's methods.

As far as my experience goes, and I have tried every method of staining that I have been able to find mentioned, these two are the only ones upon which reliance can be placed under all circumstances. With neither of them have I succeeded in finding an organism besides the bacillus of tuberculosis which would resist the decolorizing action of nitric acid, and which would not take the contrast colour. Therefore, as all the others seem to be untrustworthy from a diagnostic point of view, one of these two methods, more especially that of Koch's, should be used in all investigations upon this subject. Unless one of them is used, every observer is liable to the error of mistaking other organisms for the bacillus of tuberculosis, or to the still greater one of failing to detect it in places where proper methods of manipulation make its presence very manifest.

In all cases the greatest care should be had in regard to the purity of the staining materials employed in this, as in other work upon the micro-organisms. Reagent bottles should be thoroughly clean and new; the water used in making solutions should be freshly distilled, and the aniline salts employed should be of the very best. A little inquiry at the dye-dealers will show that this latter caution is not a useless one. Of all the colours used in microscopy there are a number of grades, denoted on the price lists by letters or numbers, *i. e.*, "Fuchsin, B.," and "Fuchsin, B.B.," etc. etc. The poorer and, by consequence, cheaper of these grades are a mixture of the refuse obtained in the manufacture of the better qualities, and are contaminated by impurities of all kinds. These impurities may of themselves form a source of error in such delicate investigations as those of which we speak. Even if the staining solution be free from impurities when freshly made, its contamination is more than likely after standing, as solutions of aniline colours seeming to offer as good a soil for the growth of bacteria as any culture medium. The writer has seen the most luxurious growth of micrococci in tuberculous lesions, which lesions were found to be entirely free from any bacteria but bacilli upon subsequent examination with fresh staining solutions; at the same time the older staining fluids were found to be swarming with the same micrococci.

Culture-methods, and Experiments.—Following Koch's directions as closely as possible, the writer has endeavoured to isolate the bacillus of tuberculosis, and to obtain a pure culture of this organism. The difficulties in the way have been very great, and to the difficulties peculiar to the circumstances under which the experiments were performed, may be

fairly ascribed the very limited number of successes when compared with the number of experiments made.

The nearest place at which a supply of blood sufficient for these experiments could be obtained involved a round trip of more than ten miles, and a corresponding increase in the chances of contamination of the blood during transportation. The method of obtaining sterilized blood-serum which was adopted was, in brief, as follows:—

Wash-bottles thoroughly sterilized by heat and plugged with cotton-wool similarly sterilized, were taken to the slaughter-houses. When the animal's throat had been thoroughly washed with a solution of corrosive sublimate (1–1000), it was opened and the blood allowed to flow into the flask, the cotton-wool plug being of course first removed. When the flask was half full, the plug was replaced, and the next flask was filled in the same manner from another animal, and so on until a supply had been obtained. Sheep's blood was used in the entire series of experiments. The blood was allowed to stand for from twelve to twenty-four hours, or until it had thoroughly coagulated, and the serum had become well separated; the latter was then removed by the following process: A glass pipette, made just before using, was drawn to a fine point at one end and sealed, the other end being plugged with cotton-wool sterilized by heat, the whole was then raised to 150° C., and after cooling was attached by a rubber tube to a Politzer's air-bag, the air being first exhausted from the bag, which was manipulated by an assistant. The point of the pipette being broken off with forceps sterilized by heat, the end was worked through the cotton plug of the flask to the serum, and the air-bag allowed to expand. In this way a very complete aspirator was obtained, and enough serum could be drawn off at a time to fill one test-tube sufficiently full. These test-tubes were first prepared in the same way as the flasks, *i. e.*, they were repeatedly subjected to a temperature of 150° C. for an hour at a time, and were plugged with cotton-wool similarly sterilized. This process of drawing off the serum is to be repeated until it is all taken up. In this way a very clear serum is obtained—better than by the method of decanting, which stirs up the blood-clot, and makes it certain that at least a portion of the serum must be thrown away from the intermingling of blood-corpuscles and colouring matter. The test-tubes were then placed in a culture-oven and raised to a temperature of 58° C. for one hour a day for seven days, this being one day more than Koch recommends, and were finally raised to 65° C., until they became solidified, the average time necessary for this to occur being about six hours. This process being successfully completed, the serum should be jelly-like, of a light amber colour, and translucent. The test-tubes should be transferred to a culture oven, and maintained at a temperature of 38° C. for a week, when, if no softening or turbidity of the serum has occurred, they may be considered ready for sowing. The test-tubes used in these experiments were about

five inches long and three-quarters of an inch in diameter, and were filled for about an inch with serum. This quantity proved to be too small, however, for the prolongation of a culture much over three weeks, by reason of the evaporation, which finally reduces the culture medium to a hard residuum. It was found impossible to prevent this evaporation with the apparatus which was at the writer's command.

Having thus obtained a culture medium suitable for the investigation of the bacillus of tuberculosis, *i. e.*, a medium that would remain solid at the temperature necessary for the growth of the organism, the obtaining of material for sowing was next in order. This material in all these experiments was obtained from the human subject, and, in all the successful ones, that is to say, in all those in which a pure culture was obtained, from the human lung. This fact, that all the material used was from the human subject rather than from animals in the laboratory, may account in a measure for the failure to obtain a larger percentage of pure cultures. It may be easily seen that the chances of contamination are very great when the material from which cultures are to be obtained must be transported from the dead-house to the work-room, and where so many delays occur as in this country are necessary before a post-mortem examination can be made. If the material is obtained from animals, the case is very different. Here the autopsy may be made at once, and in the immediate neighbourhood of the culture apparatus, and the risk of the entrance of impurities may be reduced to a minimum. Inasmuch as every delay is hazardous, and every extra manipulation opens a new channel for contamination, the difficulties in the way of procuring pure cultures from the human subject may be appreciated. Material from other organs than the lungs was employed also, but the length of time after death that elapsed before it could be used was so great, that it was found impossible to obtain pure cultures from it.

The method of obtaining culture material was essentially that of Koch, and was nearly as follows: The autopsy was made as soon as possible after death—in all successful cases within a few hours. The lungs, upon removal, were thoroughly washed in a solution of corrosive sublimate (1–1000). The hands of the operator were thoroughly disinfected by the same agency. Every cut was made with a fresh knife that had just been sterilized by heat. A portion of the tuberculous tissue the size of a small pin's head was snipped out with freshly heated scissors, or a portion of the cheesy mass was raised on the point of a platinum needle fresh from the flame. This material was then transferred to the surface of the blood-serum, the cotton-wool plug of the containing tube being removed and replaced as rapidly as possible. Rapidity in this manipulation is essential, but at the same time care should be taken to avoid too great haste, and for this reason: The author considers it possible to trace a number of failures to obtain pure cultures to over-anxiety to be quick; such over-

anxiety sometimes leading to the omission of some necessary precaution which opened the loop-hole for the entrance of other organisms.

All this being done, and the material sown being known by microscopic observation to contain bacilli, the tubes containing the inaculated blood-serum are returned to the culture oven, and kept at a temperature of 38° C. For the first few days inspection for the discovery of new growth is unnecessary, for none will appear if due to the bacillus of tuberculosis. If any change does take place during that time, the tube is to be rejected; for the change is due to other forms of bacteria.

As early as the eighth day after the inoculation of the tube the formation of light-gray very minute scales around the borders of the point of inoculation was observed, although this appearance was sometimes delayed until the tenth or twelfth day, or at least was not detected until then. In a successful experiment these scales are the first manifestation to the naked eye that there is anything going on in the tube. As the days go on the growth gradually extends itself over the surface of the serum, never, in these experiments, for more than three lines in any one direction, until at the end of three weeks it comes to a standstill, thus completing the "first generation," so called.

If the culture is proven by its gross appearances and by microscopic examination to have reached this stage free from impurities, other tubes may be inoculated by transferring a portion of the scales on the point of a platinum needle to the surface of the fresh serum and placing the whole under the same conditions of temperature as the first set of tubes. The same slow growth may be seen here as in the first case, and the cultures may be multiplied at pleasure. Under a low power lens, the peculiar growth of the scales was seen to correspond closely with the sigmoid form described and figured by Koch (*loc. cit.*), and these scales, when stained and mounted, were found to be made up of masses of bacilli full of spores. The cultures in the writer's experiments have been carried out to the fifth generation, extending over a period of four months, and in every case the peculiarities of the slow growth and dry whitish-gray scale formation have been clearly apparent.

It has not always been possible to observe the peculiar sigmoid form, because it has not always been possible to bring the scales under a low power. The blood-serum seems to lose none of its volume at the point of inoculation, and the growth seems to take place entirely upon the surface of the culture-medium. Except for the shrinking of the whole mass by drying, the volume is apparently unimpaired at the end of an experiment. The number of failures to obtain pure cultures in this series of attempts has been very large; it may be accounted for by the reasons already given and also by imperfect apparatus and the inexperience of the experimenter. The number of successes, however, was large, quite sufficiently so to demonstrate the peculiarities of the organism very clearly. Micro-

scopic preparations of "mixed cultures," that is to say, of cultures to which other organisms than the bacillus of tuberculosis had gained admittance, were obtained in large numbers and were very beautiful and instructive. These specimens showed the bacillus of tuberculosis lying side by side with various other bacteria, the latter always stained with the contrast colour. By such specimens, and more perfectly than in any other way, was it possible to bring out the peculiar contrast stain and to see how very distinct the red rods could be made to appear among masses of blue bacteria.

No attempt was made to study the growth of the organism from day to day under high powers, because of lack of time and the difficulties in the way. The sterilization of serum in a shallow dish, and the keeping it free from contamination for any length of time, is a matter of so much nicety that this part of the investigation was omitted. Cultures were examined at different stages of their growth, however, and a comparison of the condition of the organism at different stages in various cultures was thus made. In all of them the peculiarities of size and shape of the bacillus were found to be the same as have been so frequently described. In all cultures spore formation was found to be very general, the number of spores varying from two to four, often numbering five, and sometimes running up to six in a single bacillus.

Observations of the Bacillus.—As far as the observation of the writer extends, the fact of the occurrence of a peculiar form of bacillus in tuberculous lesions is an invariable one. He has never met with a case which could be considered tuberculous where he has failed to find bacilli in larger or smaller numbers. The following may be of interest, however, in this connection :—

A buck rabbit was inoculated in the anterior chamber of the eye with tuberculous material over a year before he came under observation. No result appeared at the time, except a slight inflammatory process, temporary in character. The animal remained well for over a year, and then it was noticed that the eye had become inflamed and was gradually swelling. This went on for some three months, the eye rapidly increasing in size until it reached the dimensions of half a pullet's egg, beyond the orbit. No emaciation occurred. The animal was killed, and section showed the following: The globe of the eye was normal, but was pushed forwards by a cheesy mass the size of a filbert, which filled the posterior part of the orbit completely, and was lying internally to the optic nerve. This mass was of the consistency of soft cheese, and of a grayish-white colour. In the apex of the left lung was found a nodule of a similar character, of the size of half a pea. Microscopic examination of the latter showed it to be wholly within the pleural cavity. Further examination showed the soft cheesy mass in the eye and in the lung to be made up of a large num-

ber of round cells, nuclei, and detritus, and no giant cells. The most careful search through a large number of preparations, both fresh and hardened in alcohol, failed to reveal the slightest sign of any form of micro-organism. A number of tubes were sewn with the material from this animal, but no result was obtained after weeks of waiting. As was before said, this is the only case in which there could be any question of the tuberculous nature of the process in which the writer has ever failed to discover the special bacillus of Koch. Material from other sources has been examined, as follows:—

1. Sections of tuberculous ulcer of intestine. Stained after Ehrlich. Masses of bacilli.

2. Crushed tubercle from the mesentery. Stained after Ehrlich. Masses of bacilli with many spores.

3. Miliary tuberculosis of lung. Sections stained after Ehrlich. Masses of bacilli.

4. Miliary tuberculosis of lung. Preparations stained after Ehrlich. Many bacilli.

5. Miliary tuberculosis of lung. Sections stained after Ehrlich. Masses of bacilli.

6. Miliary tuberculosis of lung, with cavities. Scrapings and sections stained after Ehrlich. Masses of bacilli.

7. Tuberculosis of long standing. Sections from the lung and kidney stained after Koch and Ehrlich contained many bacilli. Preparations of tubercles crushed between two cover-glasses, the material taken from the lung, bladder, ureter, and mesentery, and stained after Koch and Ehrlich, all showed large numbers of bacilli. Scrapings, with a freshly-heated knife, from an abscess of the kidney, a cavity of the lung, and from a sinus in the rib, which communicated with the pleural cavity, also showed the bacilli very beautifully when stained by Koch's or Ehrlich's methods. The discovery of the bacilli in the sinus of the rib is of peculiar interest as illustrating the spread of the tuberculous process into bone. In all these examinations many spores were to be seen, which would point toward considerable activity of the disease. The clinical history was found to agree with this indication, inasmuch as in the last few months of life the advance of the pathological process was seen to be very rapid.

8. Cheesy nodule in apex of lung. Sections stained after Ehrlich contained large numbers of bacilli, lying mostly toward the edges of the new formation, and extending singly into the surrounding tissue.

9. Tuberculosis of lung. Sections stained after Koch contained large numbers of bacilli.

10. Tuberculosis of larynx. Sections stained after Koch contained many bacilli.

11. Tuberculosis of lung. Crushed tubercle preparations stained after Koch showed many bacilli. Swollen laryngeal gland from the same case,

examined in sections, stained after Koch, and no bacilli were found; an interesting point, because the gland, on the strength of the clinical evidence, had been judged to be non-tuberculous.

12. Sputum, for diagnosis, stained after Koch, contained many bacilli.

13. Sputum, for diagnosis, stained after Koch, contained many bacilli.

14. Sputum, for diagnosis, stained after Koch and Ehrlich, contained many bacilli.

15. Tuberculosis of lung. Sections stained after Koch contained numerous bacilli.

16. Tuberculosis of lung. Sections stained after Koch contained many bacilli.

17. Tuberculosis of mesentery and spleen. Sections from both these organs, stained after Koch, contained large numbers of bacilli.

18. Cheesy testicle. Scrapings, stained after Koch, showed many bacilli.

19. Tuberculosis of lung. Crushed tubercle preparations, stained after Ehrlich, showed many bacilli.

20. Tuberculosis of kidney. Sections of this organ, stained after Ehrlich, contained numerous bacilli.

21. Sections of tuberculous ulcer of intestine, stained after Koch, showed many blue bacilli, together with large numbers of micrococci and other bacteria, which were stained brown.

All of this material was obtained from the human subject. The examinations are not numerous, it is true, but they are of value for these reasons: they were all made with the greatest care, and in the different steps of the preparation of the specimens, every possible precaution was taken to prevent external contamination. Staining-fluids, reagent-glasses, instruments, material, and hands were as clean and free from impurities as care could make them. Material was examined as quickly as it could be produced, and was obtained with the greatest practicable care. All the microscopic work was done with the apparatus recommended above.

Examinations yielding negative results as regards the occurrence of bacilli with the special staining reaction of the bacillus of tuberculosis have been made in a large number of cases of sputum from other diseases than tuberculosis, and in the case of the following materials, viz:—

1. Broncho-pneumonia. Sections stained after Koch and Ehrlich contained no micro-organisms of any kind.

2. Lobar pneumonia. Stained after Koch. No micro-organisms.

3. Fluid obtained from knee-joint. Stained after Koch. No results.

4. Material from a cheesy testicle, stained after Koch. Micrococci stained brown, but no other bacteria. The patient was perfectly healthy in every way with the exception of this testicle, which was swollen to about twice its normal size, and was partially broken down into a homogeneous cheesy mass.

5. Sections from a typhoid ulcer of the intestine. Large numbers of micrococci and bacilli which took the contrast stain.

6. Lobar pneumonia. Stained after Koch, and after Friedländer for the pneumonia micrococcus. Cocci answering the description of the latter were seen, but no bacilli.

7. Broncho-pneumonia. Stained in the same way as the preceding. No characteristic micro-organisms whatever.

8. Membrane from the ear (diphtheritic ?). Stained after Ehrlich. Nothing but blue micrococci.

9. Broncho-pneumonia in a child. Examined in the same way and with the same results as No. 7.

10. Gray hepatization of lung and pleurisy. Examined after Koch's and Ehrlich's methods for the bacillus of tuberculosis, no result. Examined for the pneumonia-micrococcus with success.

11. Material from the ear of a child, with signs of consolidation in the lung, stained after Ehrlich's method with a negative result. The sputum of this child at this time showed no bacilli; a later examination, however, revealed them in not very large numbers.

12. The case of the rabbit mentioned above.

The characteristics of the situation of the bacilli in these observations agree with those already given by other observers. In old cheesy nodules no bacilli were found in the centre; what were present were in general in the periphery, and extending singly into the comparatively healthy tissue surrounding the diseased process. In the smaller tubercles they were found singly and in masses throughout its extent lying between or in the interior of the cells. In general the spore formation was more abundant in those cases in which the clinical history indicated a rapid advance of the disease. The bacilli have never been observed in the bloodvessels, but occasionally in the lymph spaces.

Inoculation Experiments.—The inoculation experiments that have been made by the writer are few in number, but they have been conducted with great care.

The material for inoculation has been obtained entirely from cultures of the bacilli upon blood serum as detailed above.

The method of inoculation was purposely made as trying as possible, and was as follows: A portion of a culture so small as to be barely visible without a lens was taken up on the point of the needle of a syringe, and a drop of freshly distilled water which had just been boiled was used to liquefy it. That part of the animal selected for inoculation was first shaved and then thoroughly washed in a solution of corrosive sublimate (1–1000). A very fine puncture through the skin was then made with a freshly heated needle. This done, the drop of fluid from the needle of the syringe was forced under the skin into the subcutaneous cellular tissue,

and the wound was left to itself. The syringe used was made as nearly as possible after that described by Koch (*Mitt. a. d. Kais. Gesundheitsamte*, Bd. II. S. 60, 1884), and was thoroughly disinfected by changing the washers and by heat after each time of using. It should be needless to say that the hands of the operator were thoroughly washed in the usual solution of corrosive sublimate (1–1000) before every inoculation, and the same care was exercised in regard to everything else that came into use in the course of the manipulation. The control inoculations were performed in exactly the same way with the exception that a very much larger amount of material was injected, so that if there had been any virulence in the blood serum it would have shown itself. The same syringe was used for each series, the control inoculations being performed after the leading series, in the exact order in which they are detailed. All the animals were selected for their healthy appearance, were all males, and of ages varying from six months to a year. After inoculation they were separated and kept in a room entirely apart from any other animals, which had never been used for the same or any other experimental purpose.

Series I. *Expt.* 1.—Guinea-pig, male. Inoculated in the anterior chamber of the right eye and in the right inguinal region, with a culture from human lung; the culture was the third generation, and had been out of the lung for two months. The day after the inoculation there was a profuse iritis, the point of inoculation in the abdomen showed nothing. Two weeks later a minute nodule could be seen in the eye, and a very slight nodule could be felt in the abdomen. General condition good. A week later, appetite diminished, no emaciation apparent. For two weeks before death, diarrhœa and an enormous secretion of urine were noticed. Killed at the end of eight weeks. Section showed that the point of inoculation in the abdomen was healed, but the lymph channels radiating from it were swollen, and distinct minute nodules of a grayish colour were found in the walls of the bladder, in the vesiculæ seminales, and in the spleen. Preparations from all of these, stained after the methods of Koch and Ehrlich, showed the presence of many bacilli. The lungs and liver were normal and contained no micro-organisms; the eye showed a minute nodule on the posterior portion of the globe, and another at the external canthus. Material from both of these, stained as above, contained numerous bacilli.

Expt. 2.—Guinea-pig, male, four months old. Inoculated in right inguinal region in the same way and with the same material as the preceding. Killed in nine weeks. Section showed no appreciable disturbance of nutrition; liver much enlarged; spleen slightly enlarged, with minute grayish nodules scattered through its substance; slight obstruction beneath the point of inoculation, other organs normal. Preparations of the skin at the point of inoculation, and of the spleen, stained after Ehrlich's method, showed numbers of bacilli; none were found in the liver.

Expt. 3.—Guinea-pig, adult, male. Inoculated in right inguinal region in exactly the same manner as the preceding. Killed at the end of eight weeks, after no signs of ill-health. Section showed all the organs to be perfectly healthy. The point of inoculation could not be discovered after the most minute examination. Careful and prolonged search failed to

show any sign of any micro-organism. This experiment was, therefore, a failure.

Expt. 4.—Guinea-pig, young, male. Inoculated in the right inguinal region with the same material and in the same way as the preceding three. Killed in nine weeks. Section showed the heart and lungs to be normal; the spleen, stomach, and intestine were bound together by a mass of inflammatory tissue, which involved the supra-renal capsules and the pancreas; the small intestine, for about two and one-half inches from the pylorus, was studded with a very large number of minute, gray nodules; kidneys, bladder, and large intestine were normal. The point of inoculation was well defined, and was the seat of a minute cheesy nodule the size of a pin's head. Stained after Ehrlich's method, bacilli were found in the nodules of the intestine and in the inflammatory tissue about the stomach; none were found in the nodule at the point of inoculation, for this was lost from the cover-glass in the process of staining.

Control Inoculations. *Expt.* 5.—Guinea-pig, adult, male. Inoculated in left inguinal region, with ten minims of blood serum, softened in distilled water. The serum was obtained from a tube in which cultures of the bacillus of tuberculosis were growing, and a part of which cultures furnished the material for the previous successful inoculations; the serum was taken from a point one-half inch distant from the cultures. The animal was killed in ten weeks. Section showed absolutely nothing abnormal in the viscera; the point of inoculation was barely distinguishable. Sections stained after the methods of Koch and Ehrlich failed to show the presence of any bacteria.

Expt. 6.—Guinea-pig, male, adult. Inoculated in the same place and with the same material. Killed in nine weeks. Section showed all the organs to be perfectly healthy. The point of inoculation was entirely invisible, and microscopic examination, as before, failed to give any results.

Series II. *Expt.* 1.—Guinea-pig, adult, male. Inoculated in right inguinal region with culture from the human lung, ten weeks old. Killed in ten weeks. Section showed the point of inoculation distinct and nodular, being about the size of a pea; a cheesy degenerated patch on the posterior surface of the bladder, and a miliary nodule in the apex of the right lung; other organs normal. Under the microscope, sections of the diseased parts, stained after Koch's method, were seen to contain numerous bacilli of tuberculosis.

Expt. 2.—Guinea-pig, adult, male. Inoculated in the right inguinal region with the same culture as the preceding; killed in ten weeks. Section showed the point of inoculation to be swollen and inflamed, the size of a small pea. A deposit of miliary tubercle was found in the mesentery, and one very small nodule in one kidney. Microscopic examination of these nodules, stained after Koch, showed the presence of numerous bacilli.

Expt. 3.—Guinea-pig, adult, male. Inoculated in exactly the same manner, and with the same material as the two preceding; killed in nine weeks. Section shows the point of inoculation as a minute nodule, and there was a miliary deposit in the spleen and mesentery, with one small nodule in one kidney. In the preparations from these organs stained after Koch's method, bacilli were found in the nodules in the spleen and mesentery, but not in the kidney, or at the point of inoculation.

Control Inoculation. Expt. 4.—Guinea-pig, adult, male. This experiment was made in exactly the same manner as the control inoculations of Series I. Ten minims of blood-serum, obtained from the tube in which the cultures were growing, and but one-half inch away from them, were softened in distilled water, and injected under the skin in the left inguinal region; killed in seven weeks. Section showed every organ to be perfectly healthy; the point of inoculation was not discernible, and a large number of microscopic examinations failed to show any new growth whatever, and no bacteria.

It thus appears that of the seven animals inoculated with pure cultures of the bacillus of tuberculosis, all but one became infected with a disease similar to miliary tuberculosis. The case of failure to inoculate may be explained in different ways: by the difference in susceptibility, or by the failure to introduce the very minute portion of the culture which was employed. In every case, where any result was obtained, the characteristic bacilli were found in the youngest as well as the oldest lesions. In none of the cases were the changes so rapid or so extensive as Koch reports them to be when pure cultures of the bacilli are used; this may be due to the very minute quantity used for injection, which was purposely made as small as it was possible to handle. It is probable that if the animals had been allowed to live for a longer time the extent of the disease would have been greater; but, of course, the liability to spontaneous tuberculosis, so called, would have been greater also; the majority of the bacilli observed contained spores, and this characteristic was especially striking when the miliary nodules were present in the mesentery and intestine.

The control inoculation experiments will be granted to have been performed under as trying conditions as could have been devised, and the absence of any result, side by side with the successes with the culture, is especially striking.

Circumstances prevented the continuation of the experiments to the further culture and inoculation of the organisms found in these animals. This is to be regretted, but the abandonment of the experiments at this point was unavoidable.

The results here given, although few in number, and so, worth little by themselves, are, by reason of the care with which they were made, offered as a slight piece of confirmatory evidence in favour of the specific nature of the bacillus of tuberculosis.

Throughout this paper the organism under consideration has been called the bacillus of tuberculosis, rather than the tubercle bacillus; the reasons for this seem to the writer very obvious; the mass of observations already made, and constantly being added to, demonstrates beyond question the constant presence of a peculiar organism in all tuberculous processes. At the same time there are occasionally reported cases of cheesy degeneration in which the specific organism does not occur. A large number of these

failures to detect the organism may be ascribed to imperfection in the methods of search for it. There remains, however, a certain proportion of cases which cannot be thus explained, and in which it must be granted that the special bacterium does not exist. Here we must face the question as to whether all cheesy processes, even though making their appearance in the form of nodules, should be called tuberculous. A large part of these processes are tuberculous, and an equally large part are produced by the action of a bacillus. It seems to be only proper, therefore, to give to these processes the name of tuberculosis, and to the organism producing them the name of the bacillus of tuberculosis.

Cheesy processes in the lung may be produced by the introduction of finely divided particles, but no process extending over the whole system can be produced by these agents, as it certainly can be by the bacterium we speak of. The cheesy processes produced by indifferent substances have been repeatedly proven to have nothing specific in their nature. It is the confusion between tubercle and tuberculosis that prevents at least one observer from acknowledging the specific nature of the bacillus of tuberculosis. If the fact of the existence of a specific and of a non-specific nodule—both of them subject to cheesy degeneration—were more generally kept in mind, it would not be so very difficult to accept Koch's work as some have found it to be. Koch's work upon this subject—of the specific character of tuberculosis and the special organism connected with the process—stands to-day as unshaken as when it was concluded; and deservedly ranks as one of the most important and complete contributions to medical science of modern times.

In conclusion, it seems as if a few words of protest should be urged against the tendency to use too low powers of the microscope for the detection of the bacillus we have been speaking of. To say that a dry lens with no form of sub-stage illumination is sufficient for the detection of the stained bacilli is only true in the thinnest of fluid preparations, and then is only true in part. The assertion that such an apparatus is sufficient in all cases is absurd, and is only a proof of the untrustworthy nature of the observations made by any one relying upon it. The writer has heard of an observer who regularly makes diagnoses of the bacillus of tuberculosis with a three-quarter inch lens, and with such a power demonstrates them as red rods of about two lines in length.

No apparatus can take the place of the oil-immersion lens with a substage illuminator; the very best that the writer has seen is unquestionably the apparatus that has been uniformly employed in the work recounted in this paper, viz., a Zeiss one-twelfth homogeneous immersion-objective, a No. 3 ocular, and an Abbe's illuminating apparatus, all fitted to a No. 1 stand.

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